

## Product Information

### 4-Nitrophenyl N-acetyl-β-D-glucosaminide

Catalog Number **N9376**

Storage Temperature -20 °C

CAS RN 3459-18-5

Synonyms: NP-GlcNAc, PNP-β-D-GlcNAc,  
p-Nitrophenyl-2-acetamido-2-deoxy-  
β-D-glucopyranoside

#### Product Description

Molecular Formula: C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>O<sub>8</sub>

Molecular Weight: 342.30

λ<sub>max</sub>:<sup>1</sup> 300 nm

Extinction coefficient:<sup>1</sup> E<sup>mM</sup> = 10.8  
(at 300 nm in 0.1 M NaOH)

Specific rotation:<sup>2</sup> -15° ± 2° (5 mg/ml, water, 25 °C)

4-Nitrophenyl N-acetyl-β-D-glucosaminide (NP-GlcNAc) is used as a chromogenic substrate for β-N-Acetylglucosaminidase.<sup>1,3</sup> The cleavage product, 4-nitrophenol, is measured at 405 nm.

NP-GlcNAc can also be used in combination with diethylaminoethyl-α-cyclodextrin (DEn-CD) for a rapid and accurate rate assay method for β-N-Acetylglucosaminidase.<sup>4</sup> The cyclodextrin derivative is used as an additive to ionize 4-nitrophenol to yellow-colored 4-nitrophenoxide at a pH near 5, the pH optimum for the enzyme.

This product is synthetically prepared.

#### Preparation Instructions

This product is soluble in water (5 mg/ml). If necessary, sonication may be applied, or gentle warming at 35 °C for no more than 4 minutes. One report indicates preparation of stock solutions of this reagent at 50 mM in DMSO, although this has not been tested in our laboratories.<sup>5</sup>

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

#### Storage/Stability

Store the product at -20 °C.

#### Procedure

A rate assay method for β-N-Acetylglucosaminidase is as follows:<sup>4</sup>

1. Prepare a 4.8 mM stock solution of NP-GlcNAc and 1% DEEn-CD (n is preferably near 17) in 0.1 M glycolate buffer, pH 5.5.
2. Mix the stock solution and a properly diluted sample (urine or other body fluid) at a ratio of 1:1 in a reaction cuvette placed in a spectrophotometer and monitor the absorbance at 400–420 nm.
3. The reaction rate (enzymatic activity) can be read directly from calibration plots prepared for a given lot of DEEn-CD sample, or it can be calculated from the rate of the absorbance increase (ionization degree) of 4-nitrophenol at pH 5.5, and the millimolar absorbance coefficient of 4-nitrophenoxide in the presence of 0.5% DEEn-CD.

#### References

1. Borooah, J. *et al.*, *Biochem. J.*, **78(1)**, 106-110 (1961).
2. *Biochemical Preparations*, Volume 10, J. Wiley (New York, NY), p. 118 (1963)
3. Bowers, G.N., Jr. *et al.*, *Clin. Chem.*, **26(6)**, 724-729 (1980).
4. Shibata, H., and Yagi, T., *Clin. Chim. Acta*, **251(1)**, 53-64 (1996).
5. Sabban, S., *et al.*, *JoVE*, **93**, e52222, doi: 10.3791.52222.

CMH,RXR,GCY,HJ,MAM 10/16-1